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Environmental Consequences of Nanotechnologies

Purification and Concentration of Nanoparticles Using Diafiltration

Scientific Operating Procedure Series: SOP-P-1

Lesley F. Miller and Mark A. Chappell

July 2015



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Final report

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Abstract

Nanoparticle solutions, especially those synthesized in a laboratory, may contain additional solutes associated with synthesis and storage, such as ionic salts, suspension stabilizers, pH buffers, chelating agents, etc. These contaminants can cause instability in the nanoparticle suspensions and otherwise modify suspension behavior in a way not representative of a pure solution. Thus, it is important to purify the nanoparticle suspensions in a way that removes reactants but maintains the particles' dimensions and properties. Diafiltration is a pressure-driven filtration process that has been shown to effectively and efficiently purify nanoparticle suspensions. This report describes the diafiltration method used in the team's laboratory for controlling the initial properties of a nanoparticle dispersion. The authors wish to emphasize the importance of utilizing this technique to obtain data about the initial properties of nanoparticle dispersions. It is recommended that the data be obtained prior to endeavoring to understand how different ionic media — such as the media relevant to fate and transport experiments — impact behavior.

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Preface

This special report describes a Scientific Operating Procedure (SOP) and outlines the recommended steps to cleanse and purify nanomaterial solutions for use in laboratory experiments. The research was performed by Lesley Miller and Mark Chappell, U.S. Army Engineer Research and Development Center (ERDC) – Environmental Laboratory (EL), Vicksburg, Mississippi. The concept of using tangential flow filtration techniques for the team's purposes was developed by Spectrum Laboratories, Inc. of Rancho Dominguez, CA. Funding was provided by the Environmental Quality and Installations Program.

This study is part of the Environmental Quality/Installations (EQ/I) Research and Development Program focus area directed by Dr. Jeff Steevens. This focus area is under the direct supervision of Alan Kennedy, ERDC-EL, and under the general supervision of Dr. Elizabeth Ferguson, Technical Director for Military Munitions in the Environment, ERDC-EL. At the time this report was prepared, Dr. Jack Davis was Deputy Director, ERDC-EL, and Dr. Beth Fleming was Director, ERDC-EL. LTC John T. Tucker III was Acting Commander of ERDC and Dr. Jeffery P. Holland was Director of ERDC.

Unit Conversion Factors

Multiply	By	To Obtain
angstroms	0.1	nanometers
degrees Fahrenheit	$(F-32)/1.8$	degrees Celsius
microinches	0.0254	micrometers
ounces (mass)	0.02834952	kilograms
pints (U.S. liquid)	0.473176	liters
pounds (mass)	0.45359237	kilograms

1 Introduction

The Scientific Operating Procedure (SOP) described herein for assessing the properties of nanotechnologies was developed under Task 2: Optimized Scientific Methods of the ERDC/EL Environmental Consequences of Nanotechnologies research program. The primary goal of this Task was to develop robust SOPs for investigating the environmental health and safety- (EHS) related properties of nanotechnologies, including nanomaterials and products incorporating nanomaterials.

The present SOP describes a diafiltration procedure for removing excess solutes and stabilizing agents from dispersed nanoparticle suspensions.

The present SOP combines best laboratory practices available from the literature with the professional experience of ERDC research scientists.

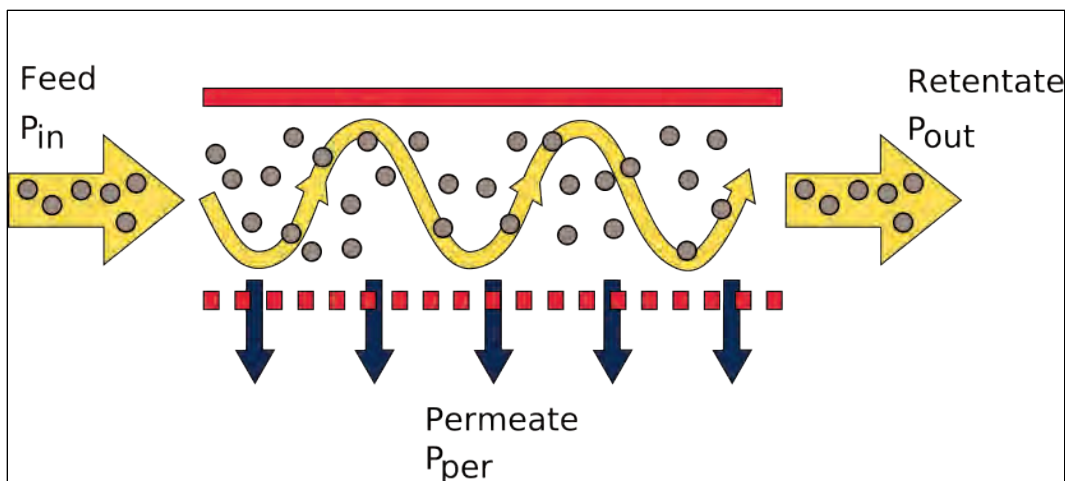
2 Background

Nanoparticle behavior in suspensions has been shown to be affected by the presence of dissolved solution components or “purity” of the suspension. Residual impurities such as reactant products or stabilizers from synthesis, can directly impact many of the chemical and physical properties of the nanoparticle suspension. For example, Sweeney et al. (2006) showed that the size distribution of platinum nanoparticles (as measured by atomic force microscopy (AFM)) was altered relative to the type and concentration of impurities. Gold nanoparticles have been observed to rapidly decompose when exposed to thiol ligand, and gold nanoparticle suspension purity also affects the self assembly of those nanoparticles shown in 1-D and 2-D arrays (Sweeney et al. 2006)(Sweeney, Woehrle and Hutchison 2006).

Many techniques have been used historically to purify nanoparticle suspensions, including centrifugation, precipitation, extraction, and dead-end filtration. Most of these traditional techniques are time-consuming and relatively inefficient. Oftentimes, synthesized nanoparticle (NP) suspensions contain reaction impurities that exhibit similar solubilities, making common filtration techniques ineffective for removing them from the suspension.

An alternate method for purifying nanoparticle suspensions is called tangential flow diafiltration (Figure 1). In essence, this technique works by “pushing” a nanoparticle suspension across a hollow fiber filter surface using a pressurized flow (Serway and Tamashiro n.d.). As the particles travel across the surface of the membrane, a small proportion of solution (approx. 10 %) and dissolved solutes permeate the hollow-fiber while the NPs are retained (the selected pore size of the hollow fiber filter depends on the NP size the user desires to retain). The retained suspension of NPs (called the retentate) is recirculated through the filtration module, with the permeate (i.e., solution and associated components that permeated the filter) replaced by a desired background solution (such as deionized water) pumped into the retentate flow from a separate delivery vessel. This process of removing and replacing background solution results in “cleaning” of the suspension by removing excess solutes. The cleaning process is continued until the desired purity is obtained. The filtrate (containing impurities from the NP suspension) is discarded.

Figure 1. Schematic of tangential flow diafiltration technique. The “P” refers to the pressure for the inlet feed, outlet retentate, and permeate.



The background buffer solution used in this SOP should be ultrapure and filtered using a 0.1 μ m filter. This ensures that dust and other unwanted particles are not concentrated through this process. Concentration of unwanted particles could interfere with analytical results for verifying the final product.

This diafiltration technique can be conducted in two different modes: constant-volume diafiltration and variable-volume diafiltration, represented schematically in Figures 2-3. Constant volume diafiltration (CVD) is a largely automated method for cleaning contaminants from nanoparticle suspensions. During CVD, the original volume of the system is unchanged throughout the process. Variable volume diafiltration (VVD) is a more labor-intensive process, where the NP suspension is simultaneously cleaned as well as concentrated. This is done by carefully setting the rate in which the permeate is replaced below the rate of recirculation. Thus, the final volume of NP suspensions after VVD treatment can be substantially less than the original volume, depending on the degree of concentration that is desired.

Figure 2. Constant Volume Diafiltration (CVD).

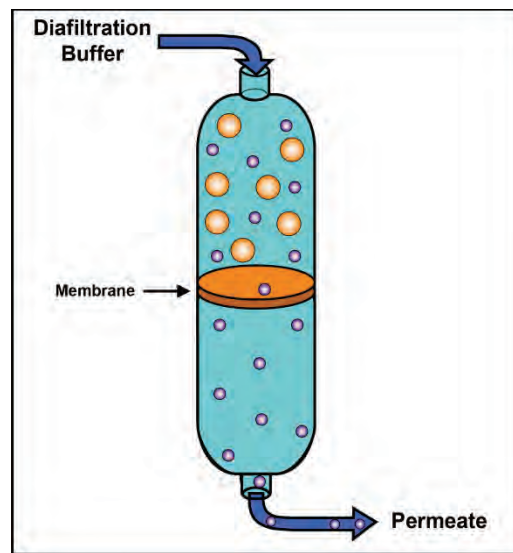
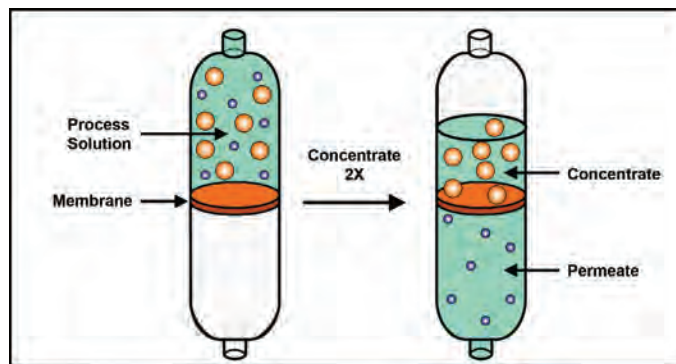


Figure 3. Variable Volume Diafiltration (VVD).

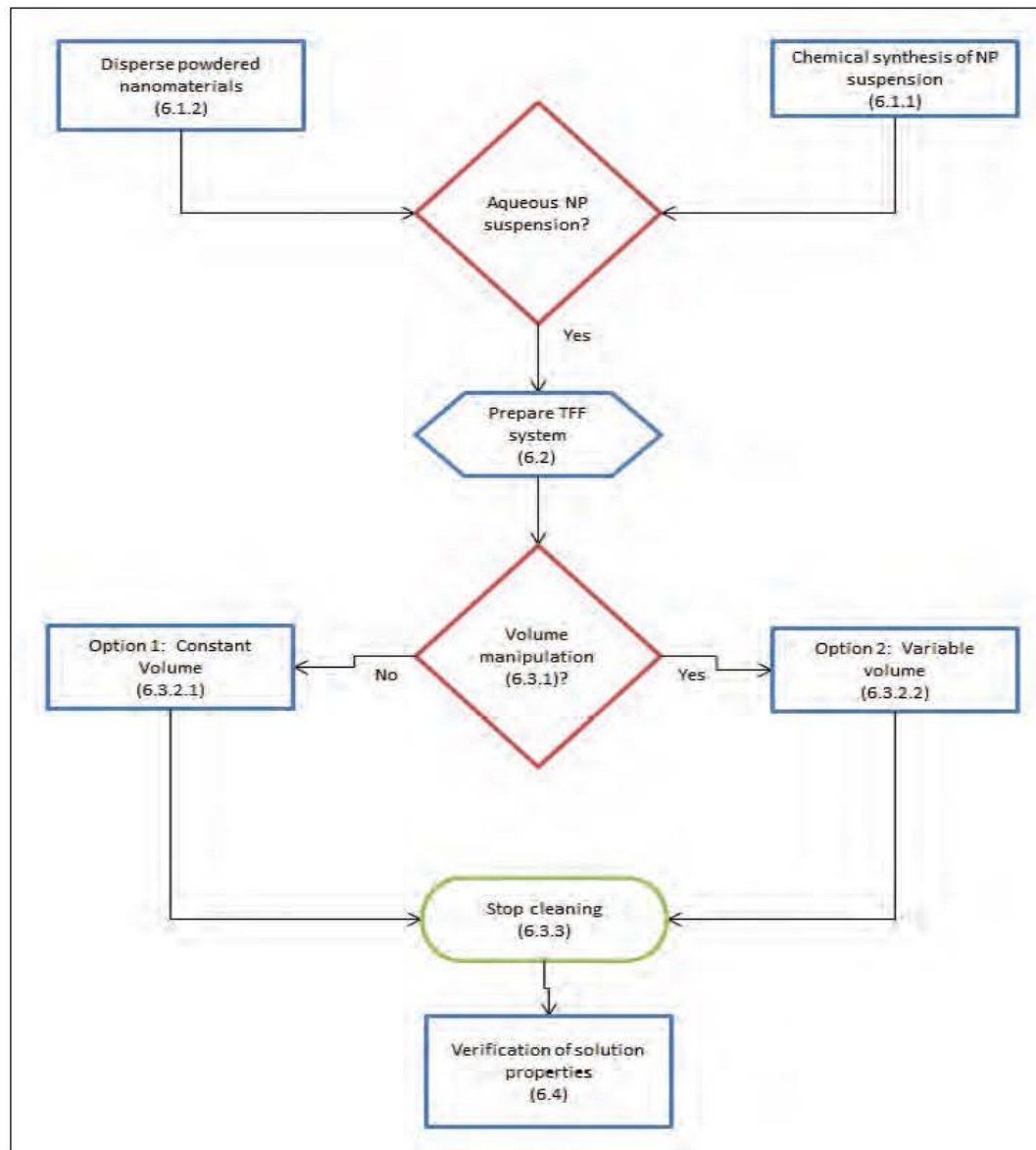


Once the user is confident that the initial NP suspension is purified, the properties of the suspension must be experimentally verified before proceeding with fate and transport studies. Verification methods should ensure that aggregation states of the suspension are not affected by the cleansing process (Rinzler et al. 1998).

3 Scope

This SOP describes a reliable method of purifying nanoparticle suspensions and properly verifying the properties of the cleansed suspension. This protocol is written for organically coated metal NP suspensions, particularly citrate-coated nanogold and nanosilver suspensions, but may be relevant to other nano-suspensions if the method is properly modified for various types and sizes of particles. NPs with other capping agents may be cleaned using this method if those capping agents do not have a tendency to adhere to the surface of the membrane. Furthermore, NP suspensions can be concentrated using this method (Figure 4).

Figure 4. Flow chart of SOP.



4 Terminology

4.1 Related Documents

- USEPA Method 6020
- ERDC/EL SR-15-1 SOP-T-1

4.2 Definitions

- Diafiltration, n—an ultrafiltration method used to remove salt and contaminants from a system where larger molecules or colloids (relative to the molecular cutoff of the column filter) are retained while the smaller molecules pass through the membrane.

4.3 Acronyms

- TFF – Tangential Flow Filtration
- ICP-MS – Inductively Coupled Plasma-Mass Spectroscopy
- DLS – Dynamic Light Scattering

5 Materials and Apparatus

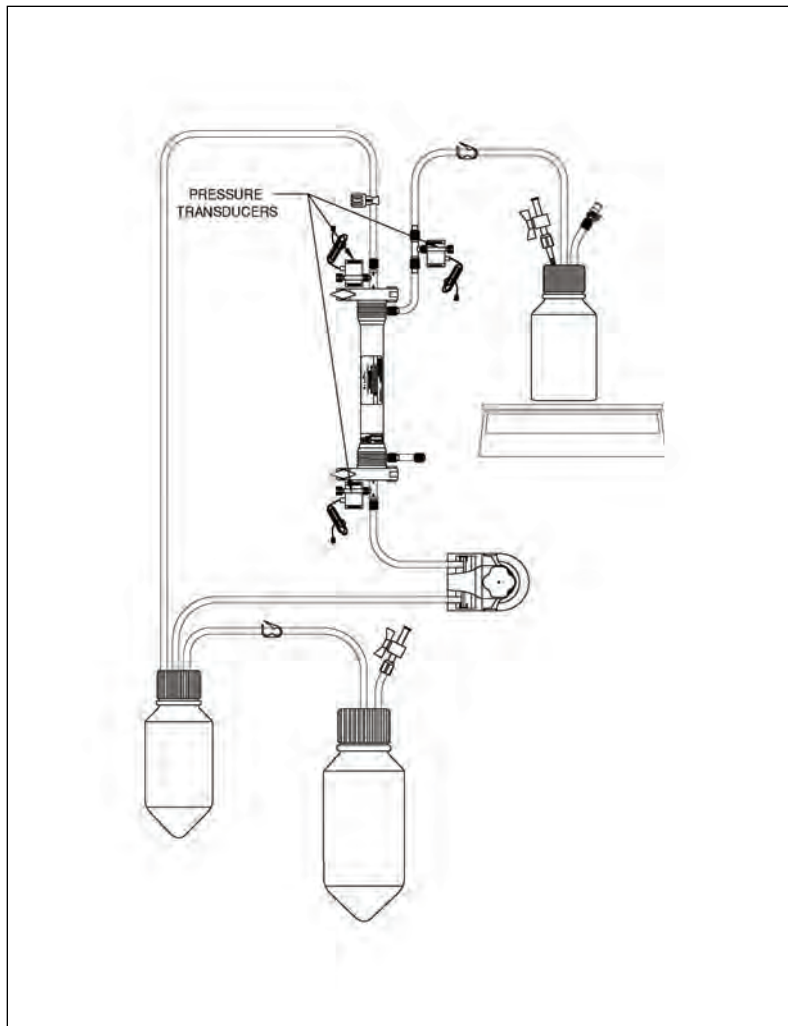
5.1 Materials

- Ultrapure deionized water
- 90mm Vacuicap™ filters (Pall®, Ann Arbor, MI) with 0.1 µm pore size
- Ultra-clean 0.075M NaOH solution (prepared with 0.1 µm filtered water)

5.2 Apparatus

- Spectrum Laboratories, Inc. Krosflo Research III TFF (Tangential Flow Filtration) system (Rancho Dominguez, CA). See Figure 5.
- Spectrum Laboratories, Inc. 30kDa MWCO polyethersulfone hollow-fiber filtration module (Rancho Dominguez, CA)
- Ohaus Scout Pro digital balance (Parsippany, NJ) or similar
- Dynamic light scattering instrument (DLS, Malvern Nano ZS)

Figure 5. SpectrumLabs KrosFlo Research III Flow Path
(SpectrumLabs n.d.)



6 Procedure

1. Synthesize nanoparticle suspension.
2. Prepare equipment for suspension cleansing and purification.
3. Conduct diafiltration.
4. Report the final particle size and concentration data.

6.1 Nanoparticle Suspension

6.1.1 Synthesize nanoparticle suspension

Nanoparticle suspensions can be laboratory synthesized using published protocols.

6.1.2 Create nanoparticle dispersion from dry NPs

Using ERDC SOP-T-1 disperse dry NPs of choice in aqueous media.

6.2 Clean and Prepare System for Filtration

6.2.1 Cleanse filter module with NaOH solution

Add 0.075M NaOH solution to filtration/processing vessel with all clamps shut. After turning on backpressure valve to TMP setting, open the clamp leading into the filtration module to allow loading of the NaOH solution into the module. Allow pressure to build to set amount, and quickly open the permeate valve. Allow solution to cleanse filter using 18 mL min⁻¹ flow rate and 9 psi transmembrane pressure for approximately 15 minutes.

6.2.2 Stop process

Close all clamps, pause backpressure valve, and open backpressure valve to stop.

6.2.3 Rinse filter module with ultra-pure DI water

Repeat Step 6.2.1 and 6.2.2 using 0.1 µm filtered water.

6.3 Purify Nanoparticle Suspension

6.3.1 Prepare system for cleansing

Place a collection flask on a tared balance. Feed permeate line to collection flask.

6.3.2 Purify nanoparticle suspension

Load buffer reservoir with 0.1 μm filtered DI water. Add desired NP suspension to filtration/processing vessel with all clamps shut. After turning on backpressure valve to TMP setting, open the clamp leading into the filtration module to allow loading of the NP suspension into the module. Allow pressure to build to set amount, and quickly open the permeate valve. Allow suspension to filter using 18 mL min^{-1} flow rate and 9 psi transmembrane pressure.

- Option 1: Constant Volume Diafiltration

Open buffer valve to allow constant volume filtration. Monitor the number of washes by monitoring the balance. A typical wash process utilizes ten washes. (i.e., a 25 mL solution is washed 10x when the volume on the balance reads 250 mL)

- Option 2: Variable Volume Diafiltration

Allow buffer valve to remain closed. When the volume of the processing vessel decreases by a desired amount, open the buffer valve to allow volume to come back up to original value. Repeat for the desired number of washes. A typical wash process consists of a loss/addition of half the volume for ten washes. After the desired number of washes is reached, allow volume to decrease to desired final amount (i.e., from 45 mL original volume to 8 mL final volume) to allow the particles to concentrate in suspension.

6.3.3 Stop processing

Repeat Step 6.2.2. Retain your nanoparticle suspension. Repeat Step 6.2.1. Close all valves to allow NaOH solution to remain in the filter module for storage.

6.4 Verification of Suspension Properties

6.4.1 Dynamic Light Scattering (DLS) analysis

Per instrument manufacturer's instructions, a one-milliliter aliquot should be injected into a clear disposable polystyrene cuvette for analysis of light scattering properties using a backscatter angle of 173 degrees. Correlograms should be analyzed to provide predominant size of particles as well as polydispersity.

6.4.2 Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis

If the nanoparticle of choice is a metal such as gold or silver, an aliquot can be measured using USEPA Method 6020 to report the final concentration of the NP in solution.

6.4.3 Electrical conductivity (EC) measurements

The EC (which roughly measures the salt content of the solution) of the permeate should be measured throughout the cleansing process. Record the permeate EC at least at every wash volume to ensure the salt concentration continues to fall during each wash process. An appropriate (within the correct electrical conductivity range) and calibrated EC electrode can be used for these measurements. Sample should continue through the cleansing process until the EC change with time is constant.

7 Reporting

7.1 Analysis of Results

Particle sizes as reported by DLS measurement should be within two standard deviations from each other, and one predominant particle size should be observed. The particle concentration (as measured by ICP) may be variable, but having this information allows one to accurately create a concentration of one's preference by dilution. The EC values should continue to decrease after each wash volume to ensure unwanted salt and contaminants are removed from the final solution.

7.2 Key Results Provided

The results from this SOP will allow the user to ensure purity of NP suspensions, and thus repeatability of specific expected properties.

7.3 QA/QC Considerations

This method is intended for use with metal-based nanoparticle suspensions. This method can be modified for other colloid types and used in conjunction with other analytical methods to verify particle characteristics. The cleansing technique should create consistently pure NP suspensions with repeatable physical and chemical characteristics, depending on the buffer and the final NP concentration chosen. Capping agents on NPs should not interfere with the method unless the capping agent is likely to adhere to the membrane surface.

References

- Rinzler, A. G., J. Liu, H. Dai, P. Nicolaev, C. B. Huffman, F. J. Rodriguez-Macias, P. J. Boul, A. H. Lu, D. Heymann, D. T. Colbert, R. S. Lee, J. E. Fischer, A. M. Rao, P. C. Eklund, and R. E. Smalley. Large-scale purification of single-wall carbon nanotubes: process, product, and characterization. *Appl. Phys. A*. 1998: 29-37.
- Serway, D., and W. A. Tamashiro. *Purification of nanoparticles by hollow fiber diafiltration*. Rancho Dominguez, CA: Spectrum Laboratories, Inc.
- SpectrumLabs. Diafiltration (buffer exchange) using hollow fiber membranes instead of dialysis tubing - automated diafiltration.
- Spectrum Laboratories, Inc. 2010. KrosFlo Research Ili TFF System. *Product information and operating instructions*. Rancho Dominguez, CA: Spectrum Laboratories, Inc.
- Sweeney, S. F., G. H. Woehrle, and J. E. Hutchison. Rapid purification and size separation of gold nanoparticles via diafiltration. *J. Am. Chem. Soc.* 2006: 3190-3197.
- U.S. Environmental Protection Agency (USEPA). 2009. Test methods for evaluation solid waste, physical and chemical methods (SW-846): Method 6020 for ICP-MS. Washington, DC: USEPA.

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